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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 9/127, 7/00

A1

(11) International Publication Number: WO 95/35095

(43) International Publication Date: 28 December 1995 (28.12.95)

(21) International Application Number: PCT/EP95/02397

(22) International Filing Date: 21 June 1995 (21.06.95)

(30) Priority Data:
08/264,204 22 June 1994 (22.06.94) US

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(81) Designated States: AU, BR, CA, CZ, FI, HU, JP, KR, LK, MX, NO, NZ, PL, RO, RU, SK, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: COMPOSITIONS FOR APPLYING ACTIVE SUBSTANCES TO OR THROUGH THE SKIN

(57) Abstract

A cosmetic or medical composition of topical application to the skin. It results in the transdermal passage of an active ingredient, or in the introduction of such agent into the skin. The essential components of such compositions are a phospholipid, a lower aliphatic alcohol of two to four carbon atoms, optionally with propylene glycol, water and a compatible active ingredient. The alcohol content is generally from 20 to 50 %, and when glycol is present, the combined percentage of alcohol and glycol being up to about 70 %. The compositions are suitable for the topical application of a wide variety of cosmetic and pharmaceutical compounds.

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DESCRIPTION

COMPOSITIONS FOR APPLYING ACTIVE SUBSTANCES TO OR THROUGH THE SKIN

FIELD OF THE INVENTION:

The invention relates to novel compositions containing phospholipids, short chain alcohols (C2-C4) and water. These compositions may also contain polyols.

Prefered compositions contain phospholipid, ethanol (EtOH), water (DDW), and propylene glycol (PG).

This invention relates to pharmaceutical, cosmetic, veterinary or phytopharmaceutical compositions for delivery to skin, membranes, or tissues, which enhance the delivery of the active agent at the site of application, where the agent may form a reservoir in the skin (membrane, tissue) or may be absorbed systemically into the blood circulation.

The compositions are hydro-alcoholic or hydro/alcoholic/glycolic phospholipid systems in which the concentration of alcohols, glycols, or their combination is relatively high. The main components of these systems are: phospholipids [with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC and others], ethanol (or other short chain alcohols), water and propylene glycol (or other glycols).

The novel composition enables the delivery of higher concentrations of active agent to/through membrane (skin). The delivery can be modulated by changes in alcohol:water or alcohol-polyol:water ratio.

State of the Prior Art

In drug delivery, there are a number of enhancing agents, some used in research and some on the market, for promoting and increasing the amount of drug delivered to/through the skin. Exapmles of these enhancing agents are: DMSO, pyrrolidone derivatives, n-decylMSO, some surfactants, oleic acid, ethanol, Azone^{R}, and others(Barry, 1989).

Phospholipids are known for their broad use in liposomal systems as well as emulsifiers in the preparation of emulsions. All these systems used for pharmaceutical or cosmetic purposes are aqueous systems

with small if any concentration of alcohol and/or glycol for preservation and/or improving texture of the formulation. Emulsions containing phospholipids are prepared by mixing an aqueous and an oily phase, in some cases followed by use of an homogenizer.

Preparation of liposomal systems involves the use of organic solvents such as chloroform, alcohols and others. The prior art teaches away from high concentrations of alcohol in the final liposomal preparations. In some methods of preparation, an organic phospholipid solution is evaporated to form a lipidic film, which is then hydrated to give an aqueous vesicular system(Riaz et al.,1988). In alternative methods, liposomes are prepared by injecting an ethanolic solution of lipid into an aqueous solution, resulting in a dilute ethanolic solution (2.5-7.5% ethanol)(Batzri et al.,1973) or by dilution of proliposomes (Leigh, 1991). The alcohol, is then removed by different means such as dialysis (Kremer et al, 1977) or is diluted. The alcohol, if present is in low concentrations only, less than about 20% in the final product (e.g. 7.5%, Kremer et al, 1977; Leigh,1991).

Brief Description of the Invention

The invention relates to compositions which are hydro/alcoholic or hydro/alcoholic/glycolic phospholipid systems in which the concentration of alcohol or its combination with polyol is relatively high. We call these compositions: Ethosomal systems. The systems described in this invention are pharmaceutical, cosmetic, phytopharmaceutical or veterinary compositions for application to the skin (or other tissues) comprised of combinations of phospholipids, alcohols, water and glycol (polyols), as major components. An ethosomal system is a skin permeation enhancing system which has the potential to form ethosomes, which are "soft" vesicles formed from phospholipid in the presence of water and ethanol (alcohol) and sometimes glycols (polyols). The size of the vesicles depends on the water: alcohol ratio (see Tables 3-5) and on the phospholipid concentration. On the skin, the ethosomes change their size by fusing together as a result of the change in solvent ratio. In the preparation, the vesicle size does not change since the ratio between the solvents is constant. Penetration and evaporation of the alcolhol following application to the skin allows the transition from small to large vesicles, which grow in size until a film is formed.

An important characteristic of ethosomes is enhanced membrane permeability for various compounds. Ethosomal systems , vesicular in

nature, depending on the ratio of the components and the chemical structure of the phospolipids, can be comprised of very small entities (nm's) up to larger vesicles (mm's) (see Tables 3-5). High alcoholic (organic solvent) concentration favors the production of ethosomes in nm's range while high aqueous and phospholipid concentrations favor the formation of large size ethosomes. As examples, formulation 509(Table 4) containing 60% organic solvent and 38% water has a mean population of tens of nm's, while formulation 510 containing 50% organic solvent and 48% water has a mean population of 1mm. In system 509 the concentration of ethanol was 48% while in formulation 510 the ethanol concentration is only 20%., showing that the alcohol concentration is of great importance in determining vesicle size. The phospholipids which can be used are: phosphatidylcholine (PC), phsophatidylserine (PS), phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylglycol (PPG), hydrogenated PC and others. Some prefered phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). The concentration of phospholipid ranges between about 0.5-10% w/w. Cholesterol at concentrations ranging between about 0.1-1% can also be added to the preparation. Examples of alcohols which can be used are: ethanol and isopropyl alcohol. Examples of glycols are propylene glycol and Transcutol^{R}. The source of the phospholipids can be egg, soybean, semi-synthetics, and synthetics. Non ionic surfactants can be combined with the phospholipids in these preparations e.g. PEG-alkyl ethers (Brij-52). Cationic lipids like cocoamide, Poe alkyl amines, dodecylamine, cetrimide, and like. The concentration of alcohol (EtOH etc.) in the final product ranges from about 20-50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between about 22 to 70%. The rest of the carrier contains water and possible additives. Vesicle formation is dependent on the water: alcohol ratio. This ratio is kept constant in the product, therefore, no changes in the entities population occur. Nevertheless, penetration and evaporation of the alcohol following application to the skin allows the transition from small vesicles to a larger ones, finally resulting in film formation. In contrast to the present state of the art where "tough" liposomes accomplished by addition of different substances like cholesterol to the phospholipids and in abssence of alcohol, this invention relates to "soft" vesicles, that can be easily formed in a hydroalcoholic medium. One of the important properties of these systems is that small entities can penetrate into the skin, while larger vesicles can form a reservior

in the skin and a film on the skin surface as a result of solvent evaporation taking place after the application.

These carriers can be used to deliver various active agents such as: peptides, anti-aging, tanning agents, , vitamins, antiviral drugs, psoriasis treatment agents, hormones, medicinally active componenets of plants such as oleoresins, volatile oils, glycosides, alkaloides, terpenes and others.

Examples of other active agents which can be used with these systems are as follows: drugs like nicotine, nitroglycerine, estradiol (or like), testosterone (or like), progesterone, nifedipine, minoxidil, tetrahydrocannabinol (THC) or other cannabinoids, xanthines, anxiolytics (diazepam and others), antiepileptic (valnoctamide and others), diclofenac (and other NSAIDs),antibiotics, corticosteroids, tocopherol, 5-FU, acyclovir, colchicine, prazosin, papaverine, miconazole nitrate, ketoconazole and other antifungals, enzymes like SOD; peptides and amino acids.

Methods

In vitro skin penetration/permeation measurements

Skin permeation experiments were carried out in our laboratory as previously described (Touitou, 1986; Touitou and Fabin, 1988).

Full thickness skin

The animals used in these investigations were hairless mice obtained from the Weizmann Institute, Rehovot, Israel. The skin of 5-7 week old male mice was excised before the experiment, examined for integrity and cut for diffusion cell assembly. In most of the experiments, the skin from the abdominal site was used.

In vitro permeation experiments in horizontal diffusion cells.

The Valia-Chien cell assembly was used to perform the experiments on permeation of the drugs from various donor compositions through hairless mouse skin and the concentration in the skin. Mouse skin treated as described above was mounted in cells with a surface area of 0.64 cm^2 and half-cell volume of 3 ml. The receiver compartments contained a medium (hydroalcoholic, water, or buffer solutions) for ensuring pseudo-sink conditions. During the expeirment, samples were withdrawn and mixed with scintillation cocktail (Packard, USA) and assayed in the Kontron Betamatic Scintillation Counter (Lumitron Scientific Industries) or directly assayed by HPLC. The sample volumes were replaced with fresh solution. The results were treated using the "Transderm" computer program (Touitou and Fabin,1988). Each experiment was tetraplicated. The two-tailed, paired Wilcoxon test was used for determining the statistical significance of the effect of enhancers on the permeability coefficient changes. For these analyses, the "Balance" (IBM) computer program was used.

Skin Permeation Determination in Franz Cells

The permeation flux of drugs and the quantity of drug in the skin from the test system was measured in vertical cells (Franz diffusion cells). The experimental system conditions were: receiver temperature 37^0C and receiver volume 4-8 ml. The samples were assayed either by scintillation counting or by HPLC. The results are presented as a mean of 4 experiments and were analyzed by Transderm and by Balance (IBM) computer programs.

Size Distribution of Liposomes:

The size distribution of ethosomes was measured in a Malvern autosizer (Malvern). (See Tables3-5).

Method of Preparation and Compositions:

Examples of compositions are given in Tables 1 and 2.

a) Ethosomal system containing a relatively high concentration of Ethanol or Ethanol and Propylene Glycol was obtained as follows:

A lipophilic drug(10%) and PL-90(5%) were dissolved in an EtOH-PG(62.4%) mixture at room temperature. DDW(22.6%) was then added with vigorous stirring.

See example II-Immune agent.

b) Ethosomal systems containing a relatively high concentration of Ethanol (20-50%) or Ethanol and Propylene Glycol (22-70%) were obtained as follows:

PL-90 (0.5-10%) was dissolved in the EtOH-PG mixture. Hydrophilic drugs were dissolved in DDW and added to the PL-90 solution.

c) Ethosomal system was prepared by mixing (Heidolph mixer) PL-90 and water in concentrations as in "b" and heating to dispersion at 60-70^{0}C . The dispersion was then cooled (ice bath) with constant mixing for 30 minutes. To the above dispersion a solution of 2% Minoxidil in ethanol-propylene glycol (concentration as above) was added with vigorous mixing. A vesicular system was obtained. The preparation may be passed through a homogenizer (like Gaoline).

d) Minoxidil ethosomes were prepared by gently heating or at room temperature, a solution of soybean lecithin (Phospholipon 90) and Minoxidil in a propylene glycol ethanol mixture. Distilled water or buffer solution was added to the above system. A vesicular system was formed. The preparation may be passed through a homogenizer (like Gaoline). concentrations of ingredients as in "c".

e) A vesicular system containing a relatively high concentration of Ethanol or Ethanol and Propylene Glycol was obtained as follows: A dispersion containing soya phospholipid (Phospholipon 90), Minoxidil, Ethanol, propylene glycol, double distilled water or buffer solution, is passed through a homogenizer in order to reduce particle size. concentrations of ingredients as in "c".

f) Composition and preparation as described in a-c but containing 0.2 % cholesterol in addition.

g) As in a-d where mixtures of phospholipids are used.

h) The vesicular systems can be incorporated in various carriers such as: PVP/VA (gels, membranes, solutions), PVP (gels, membranes, solutions) carbomer gels, polaxomer (gels, solutions), emulsions, adhesives, creams, Pluronic F127 or Tetronic gels and the like, cellulose derivatives gels, plant extract gels (aloe vera gel etc), and the like.

Table 1: Examples of skin permeation enhancing systems containing various drugs

	Systems									
	DYPH1	DYPH2	MND200	SOD Immune	TH-IC 1	MM33	MM39	MM42	MM43	
COMPONENTS										
DYPHYLLINE	1%	1%								
ACYCLOVIR										
DICLOFENAC			7μCi/ml							
SOD				6%						
ROQUINIMEX					10%					
MINOXIDIL										
TH-IC						2%	2%	2%	2%	
					7μci/ml					
PL-90	5%	5%	2%	2%	5%	2%	2%	2%	.5%	5%
CHOLESTEROL	-	-	-	-	-	-	-	-	-	0.1%
PG	18.8%	-	19.6%	18.4%	31.2%	19.6%	19.2%	19.2%	19.5%	18.6%
DDW	47%	47%	49%	46%	22.6%	29.4	28.8%	48%	48.8%	27.9%
EtOH	28.2%	47%	29.4%	27.6%	31.2%	49%	48%	28.8%	29.3%	46.5%

Examples of compositions and preparation methods:

Example I- SOD ethosomal preparation

A)	Amerchol L-101	57.1%	4 parts
	Amerlate P	14.3%	1 part
	Brij 52	28.6%	2 parts
B)	Carbopol 934P	1%	
	DDW	98%	
	TEA	1%	
	SOD	6%	
C1)	PL-90	2%	
	DDW	46%	
C2)	EtOH 3	27.6%	
	PG	18.4%	

Gel preparation:

1. Preparation of "A" by melting the ingredients in a water bath. Cool while stirring.
2. Disperse Carb 934 P in DDW at room temperature.
3. Mix A and B in the ratio of 7% of A with 93% B and add TEA.

System preparation:

4. SOD was dissolved in a PL-90 dispersion in DDW [obtained by heating in a water bath of 70^0C with constant mixing (C1)].
5. An EtOH:PG mixture (C2) was prepared and added to C1 with vigorous mixing and cooling in an ice bath.

Final formulation:

6. Mix gel with the system in a 1:1 ratio.

Drug	3%
Lipid phase (A)	3.5%
Gel (B)	46.5%
PL-90 + DDW (C1)	22%
PG + EtOH(C2)	25%

Example II- IMMUNE AGENT (ROQUINIMEX) ethosomal preparation

A) Pluronic F127	40%
DDW	60%
B) ROQUINIMEX	10%
PL-90	5%
EtOH 95%	31.2%
PG	31.2%
DDW	22.6%

Prepare a Pluronic gel of 40% in DDW. Dissolve Roquinimex and PL-90 in the EtOH-PG mixture. Add DDW to the latter with vigorous stirring.
Add B to A with vigorous stirring, at a ratio of 1:1.

Example III- THC Ethosomal preparation

THC	1%
PL-90	4.2%
EtOH	51.7%
PG	15%
PVP-VA S-630	12.5%
DDW	16.6%

THC and PL-90 were dissolved in an EtOH-PG mixture with gentle heating while mixing. DDW was added with continuous stirring. PVP-VA was slowly added while stirring. The preparation was sonicated in 3 cycles of 5 minutes with 5 minutes rest between each cycle.

Example IV- Minoxidil ethosomal preparation (MM50-G)

A)	Carbopol 934P	0.75%
	Ethomeen C/15	0.75%
	DDW	73.5%
	EtOH	25%
B)	Minoxidil	4%
	PL-90	5%
	EtOH 95%	40%
	PG	20%
	DDW	31%
	Tocopherol	0.02%

MM50-G, Final concentrations:

Carbopol 934 P	0.38%
Ethomeen C/15	0.38%
Minoxidil	2%
PL-90	2.5%
EtOH (95%)	32.5%
PG	10%
DDW	52.3%

Carbopol gel was prepared by dispersing Carbopol 934P in DDW and Ethomeen was added. Ethanol was added to obtain an hydroalcoholic gel. The drug, antioxidant, and PL-90 were dissolved in the EtOH -PG mixture with gentle heating (30^0C). DDW was added with vigorous stirring to obtain the system.

The system may be homogenized.

B was added to A at a 1:1 ratio.

The mean size of vesicles was found to be 35nm

Example V- Minoxidil ethosomal preparation (MM)

As in example IV (MM50-G) with the following variations:

- The gel:system ratio may be changed to 1:2, 1:3, or 1:4.
- The ratio between solvents can be changed: EtOH (25-49%), PG (0-20%), and DDW (25-49%)can be changed.
- The PL-90 concentration can be raised to about 10%.

Example VI- DICLOFENAC SODIUM ethosomal preparation

Diclofenac sod.	1%
PL-90	1%
Carbopol 934	0.9%
Ammonium 10% soln.	1.8%
EtOH	21.9%
DDW	68.9%
PG	4.16%

Example VII- ACYCLOVIR ethosomal preparation

Acyclovir	5%
Ammonium 10% soln.	1.66%
Carbopol 934	0.83%
PL-90	2.5%
DDW	58.3%
EtOH	27.5%
PG	4.16%

Table 2: Other Examples

Sys. No.	Ingredients, % w/w					Method*
	PL-90	DDW	EtOH	PG	Other	
500	2	48	30	20		Hot
501	2	48	30	20		Cold
502	2	30	48	20		Hot
503	2	30	48	20		Cold
504	5	45	30	20		Hot
505	5	45	30	20		Cold
506	5	30	45	20		Hot
507	5	30	45	20		Cold
508	2	30	38	30		Cold
509	2	38	30	30		Cold
510	2	48	20	30		Cold
511	1.8	30	38	30	0.2(M(-)*)	Cold
512	5	30	45	20		Cold
513	5	35	30	30		Cold
514	2	33	30	30	5(Brij52)	Cold
515	17	26	40	17		Cold
516	9.3	44.4	27.7	18.5		Cold
517	5	20	40	40	5(Brij52)	Cold
518	2	47	30	20	1(Col**)	Cold
525	10	64	26	0		Hot
529	1.7	55.4	34.3	8.6		Cold

PL-90: phospholipid; DDW: water; Etoh: ethanol; PG: propylene glycol
*M(-): l-Menthol
**Col: Colchicine

* See "Hot" or "Cold" methods described below.

"Cold" method:

Phospholipid is dissolved in ethanol at room temperature by vigorous stirring with the use of Heidolph mixer. Propylene glycol is added during the stirring. The mixture is heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture which is then stirred for 5 minutes in a covered vessel.

"Hot" method:

The phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°C the organic phase is added to the aqueous one.

During the preparation process the drug is dissolved in water or in ethanol depending on its hydrophilic/hydrohpobic properties.

EXAMPLES OF SKIN PERMEATION PARAMETERS OF VARIOUS DRUGS FROM THESE SYSTEMS (Kp= permeability coefficient; Qs = quantity of drug in the skin at the end of the experiment)

Kp reflects the permeation through the skin and Qs reflects the reservoir formation in the skin.

Example VIII

MM33= MM36 (see Table 1)	vs.	blank MM (Minoxitrim^R, Trima, Israel) (2% minoxidil)
Kp= 1.36 x 10^{-2} cm*hr^{-1}		8.84 x 10^{-4} cm*hr^{-1}
Qskin=0.658 mg/cm^2		0.0479 mg/cm^2

The ethosomal system showed an increse of 15.4 times in Kp and 13.7 times in Qs.

Example. IX

MM39 (see Table 1)	MM blank(as above)
Kp=1.96 x 10^{-3} cm*hr^{-1}	5.75 x 10^{-5} cm*hr^{-1}
Qskin =0.138 mg/cm^2	0.0378 mg/cm^2

The ethosomal system showed an increse of 34 times in Kp and 3.7 times in Qs.

Example X

THC (as in ex. III)	THC in PG:EtOH (1:1)
Kp =7.2 x 10^{-3} cm*hr^{-1}	2.03 x 10^{-3} cm*hr^{-1}

The ethosomal system showed an increse of 3.5 times in Kp. No significant changes in other kinetic parameters were observed

Example XI

MM50G (as in ex. IV)	MM blank
Qreceiver =0.135 $\mu g/cm^2$ (24 hours)	0.023 $\mu g/cm^2$

The ethosomal system showed an increase of 5.9 times in Kp

Example XII

	ACV11 (as in ex VII)	Zovirax^R
Qreceiver (6 hours)	2.9 $\pm$ 1.57 $\mu g/cm^2$	3.065 $\pm$0.38 $\mu g/cm^2$
Qskin	81.35 $\pm$ 5.58 $\mu g/cm^2$	18.35 $\pm$ 8.56 $\mu g/cm^2$

The ethosomal system showed no significant change in Kp and an increase of 4.4 times in Qs.

Example XIII

MM122 2%PL-90, 2% Minoxidil 48% EtOH 95, 28% DDW, 20% PG	MM122 blank a 2%PL-90, 2% Minoxidil in EtOH	MM122 blank b 2% Minoxidil in EtOH
Qrec=3672 $\pm$ 378 $\mu g/cm^2$ (24 hours)	217.5$\pm$ 118.5$\mu g/cm^2$	66.9$\pm$22.1$\mu g/cm^2$
Qskin =570 $\pm$ 130.5 $\mu g/cm^2$	139.1 $\pm$ 25.3 $\mu g/cm^2$	53.5 $\pm$12 $\mu g/cm^2$

The ethosomal system showed an increase of 17 times in Kp and 4.1 times in Qs relative to a 2% phospholipid solution in ethanol (blank a) and an increse of 55 times in Kp and 11 times in Qs relative to an ethanolic solution (blank b). These results clearly indicate that the ethosomal system is a much more effective penetration enhancing system than ethanol or an ethanolic solution of phospholipid.

Example XIV

	MND200	Blank MND (only solvents)
Kp =	4.27 x 10^{-3} cm*hr^{-1}	1.57 x 10^{-3} cm*hr^{-1}
Qskin =	2.75 x 10^{-5} mg/cm^2	2.35 x 10^{-5} mg/cm^2
lag time =	0:41 hrs	2:30 hrs

The ethosomal system showed an increase of 2.7 times in Kp no significant change in Qs and a decrease of 5 times in lag time.

Example XV

Differential Scanning Calorimetric (DSC) data on a system containing 5% PL-90 in water and an ethosomal system No. 525 (see Table 2 for exact composition) are presented in Figs.1 and 2, respectively. The thermograms clearly show a decrease in Tm (transition temperature) value for the ethosomal system which indicates an increase in phospholipid vesicle's fluidity.

Example XVI:

Figures 3 and 4 present photographs of vesicles in ethosomal systems 510 and 529 (Table 2) as seen by means of a computerized image analyser connected to a light microscope (Cue 2 Galai- Axioscope Zeiss). These photos clearly show the presence of vesicles (ethosomes) in systems containing 20 and 34.3% ethanol, respectively.

Example XVII

There were prepared: Caffeine ethosomes (liposomal systems containing 20.9 and 35% ethanol) versus Caffeine liposomes with 5% ethanol.

All three preparations contain the same concentration of caffeine (3%) and the same phospholipid (Phospholipon 90) at 5% concentration, without propylene glycol.

Methods

In vitro skin penetration/permeation measurements.
Skin permeation experiment was carried out in our laboratory by using the Valia-Chien cell assembly to perform the experiment.

The skin of 5-7 week old male mice was used in this investigation (nude mice obtained from the Weizmann Institute, Rehovot, Israel.). The skin was examined for integrity and cut for mounting on diffusion cell assembly. The skin from the back site was used.

The skin was mounted in cells with a surface area of 0.64 cm^2 and half-cell volume of 3 ml. The receiver compartments contained water for ensuring pseudo-sink conditions. Samples were directly assayed by HPLC (Touitou et al, 1994). The experiment was run for 24 hours.
Each formulation was tested in three cells.

Caffeine Ethosomes

A)	Caffeine	3.0%
	Sod. Salicylate	4.8%
	Distilled Water	52.2%

B) Phospholipon-90	5.0%
Ethanol	35.0%

Example XVIII

A composition was prepared as set out on page 6, b.

A) Dissolve Caffeine and Sod. Salicylate in the water.

B) Dissolve the phospholipid in ethanol. Add A to B with vigorous stirring.

Caffeine Liposomes

Caffeine	3.0%
Sod. Salicylate	4.8%
Phospholipon-90	5.0%
Ethanol	5%
Distilled Water	82.2%

Preparation as above.

Results

Q_{rec} = quantity of drug in the receiver compartment of the diffusion cell at the end of the experiment.

Q_{rec}

Caffeine Liposomes (5% ethanol)	Caffeine Ethosomes (20.9% ethanol)	Caffeine Ethosomes (35% ethanol)
86.8±17.1 μg/cm2	496.6±71 μg/cm^2	4794.3±812 μg/cm2

These results clearly indicate that the ethosomal system according to the invention containing 35% ethanol enabled an enhanced delivery of caffeine through the skin of 53 times higher than the liposomes containing caffeine with 5% ethanol. The above proves the outstanding improvement resulting from a high content of alcohol in the liposomal system, in the presence of liposomes.

An increase of skin penetration from 87 $\mu g/cm^2$ to about 4794 $\mu g/cm^2$, i.e., an increase by a factor of about 53 times as large, demonstrates a dramatic and unexpected result of the novel liposomes with a high ethanol content, termed "ethosomes". A 53-fold skin penetration could not be expected at all on the basis of the prior art, which clearly teaches away from the present invention, i.e. that a high ethanol content is detrimental for liposomal preparations, and that the ethanol content of the final liposome preparation ought to be reduced so as to remove a large part of the initial ethanol content or by dilution.

Example XIX

Further experiments were carried out with Minoxidil, comparing liposomes with a high ethanol content with Minoxidil in the vehicle.

The experiments were carried out as follows:
The formulations tested were: Minoxidil ethosomes (liposomal systems containing ethanol) versus Minoxidil in vehicle. Both preparations contain the same concentration of Minoxidil (1%).

Methods

In vitro skin permeation measurements:
Skin permeation experiment was carried out in our laboratory by using the Franz cell assembly to perform the experiment.

Frozen back side skin of a 5-7 week old male mice was used in this investigation (nude mice obtained from the Weizmann Institute, Rehovot, Israel). The skin was examined for integrity and cut for mounting on diffusion cell assembly.

Nude mouse skin was mounted in cells with a surface area of 1.77 cm^2 and receiver volume of about 7 ml. The receiver compartments contained 1/150M pH7 phosphate buffer for ensuring pseudo sink conditions. Samples were directly assayed by HPLC. The experiment was run for 12 hours. Each formulation was tested in three cells.

Minoxidil Ethosomes

Minoxidil	1%	
Phospholipon	2%	
Ethanol 95%		40%
Distilled Water	57%	

Minoxidil vehicle

Minoxidil	1%	
Ethanol 95%		40%
Distilled Water	59%	

Results

Q_{rec}= quantity in the receiver compartment of the diffusion cell at the end of experiment.

Q_{rec}	
Minoxidil vehicle	Minoxidil ethosomes
16.26±2.8 μg/cm^2	64.02±22.5 μg/cm^2

The above results, of about 16 $\mu g/cm^2$ versus about 64 $\mu g/cm^2$ skin penetration of the two preparations, demonstrates that the "ethosomes" of the invention resulted in an about 4-fold skin penetration compared with the penetration of the active substance in the vehicle only, i.e. not in liposome form. These ethosomes were without propylene glycol.

Example XX

The following experimental results, relate to various liposome systems of the invention containing 1% sodium diclofenac as model drug and in which various compositional factors have been changed: 1. the concentration of alcohol 2. the phospholipid 3. the type of alcohol. The results demonstrate: 1. the cruciality of high concentrations of alcohol, and that the high skin permeation from ethosomal systems of the invention is still obtained: 2. with an additional example of phospholipid (Lipoid E 75-containing phosphatidyl ethanolamine and phosphatidyl choline isolated from egg, produced by Lipoid KG; Germany, 3. with isopropyl alcohol.

Methods:

In vitro skin permeation measurements.

Skin permeation experiment was carried out in our laboratory by using the Valia Chien assembly to perform the experiments.

Frozen back side skin of a 5-7 week old mouse was used in these experiments (nude mice obtained from the Weizman Institute, Rehovot, Israel). The skin was examined for integrity and cut for mounting on diffusion cell assembly.

The skin was mounted in cells with a surface area of 0.64 cm^2 and receiver volume of about 3 ml. The receiver compartments contained 1/150M pH7 phosphate buffer for ensuring pseudo sink conditions.

Samples were directly assayed by HPLC. The experiments were run for 17 hours. Each formulation was tested in triplicates.

FORMULATIONS:

	XA	XB_1	XB_2	XC	XD	XE
			% w/w			
Sod.Diclofenac	1	1	1	1	1	1
Phospholipon 90	5	5	5	-	-	5
Lipoid E	-	-	-	5	5	-
Ethanol	5	21	35	5	35	-
Isopropyl Alcohol	-	-	-	-	-	35
Distilled Water	89	73	59	89	59	59

Results:

Q_{rec}= quantity in the receiver compartment of the diffusion cell at the end of experiment.

Q_{rec}, $\mu g/cm^2$

XA	XB_1	XB_2	XC	XD	XE
37.7± 11	144.1±14	309.2±29	57.6±25	285.2±37	1558.8±778

These results clearly indicate that:

1. The ethosomal systems of the hydrophilic drug diclofenac sodium, containing a high concentration of ethanol (35%), give an

enhanced delivery of drug through the skin of eight times higher than the preparation containing only 5% ethanol;

2. The enhancing effect is also obtained with isopropyl alcohol;

3. The enhancing effect is obtained when the composition of phospholipids is changed;

The above results demonstrate the improvement in permeation with novel liposimal ("Ethosomal") systems of the invention.
Remark: all these systems are without propylene glycol (PG) showing the noncriticallity of PG.

The invention is illustrated with reference to the enclosed Figures and photos, in which:
Fig. 1 is a graph showing Differential Scanning Calorimetric data of an ethosomal system containing 5% PL-90 in water.
Fig. 2 is a graph showing Differential Scanning Calorimetric data of ethosomal system No. 525 (See Table 2).
Fig. 3 is a photograph of ethosomal systemt No. 510 (See Table 2).
Fig. 4 is a photograph of ethosomal system No. 529 (See Table 2).

TABLE No. 3

MALVERN AUTOSIZER II c for ethosomal system No. 501 (see Table 2)

Sample: 501; Vortex t = 22.6; Aperture: 200
Data from accumulation file sum of 5 blocks
Temperature: 22.6°C; Viscosity: 4.1800; Refractive Index: 1.365;
Angle: 90.0

```
--------------------------------------------------------------------------------
Particle size distribution ( nm. ) Graph of Distribution of mass
   10.0  -   12.1  |+
   12.1  -   14.6  |+
   14.6  -   17.7  |+
   17.7  -   21.5  |+
   21.5  -   26.0  |+
   26.0  -   31.5  |+
   31.5  -   38.2  |+
   38.2  -   46.2  |+
   46.2  -   56.0  |+
   56.0  -   67.9  |+
   67.9  -   82.2  |+
   82.2  -   99.6  |+
   99.6  -  120.7  |+
  120.7  -  146.3  |--+
  146.3  -  177.2  |+
  177.2  -  214.7  |-+
  214.7  -  260.1  |---+
  260.1  -  315.1  |--------+
  315.1  -  381.7  |----------------------+
  381.7  -  462.5  |--------------------------------------------+
  462.5  -  560.3  |----------------------------------------------.
  560.3  -  678.8  |-----------------------+
  678.8  -  822.4  |----------+
  822.4  -  996.4  |--------+
--------------------------------------------------------------------------------
```

TABLE No. 4

MALVERN AUTOSIZER II c for othosomal system No. 509 (see Table 2)

Sample: 509; Vortex + US; Non diluted
Data from accumulation file sum of 8 blocks
Temperature: 22.6°C; Viscosity: 4.8200; Refractive Index: 1.376;
Angle: 90.0

Particle size distribution (nm.)			Graph of Distribution of mass
4.5	-	5.4	+
5.4	-	6.4	----------+
6.4	-	7.7	----------+
7.7	-	9.2	----------+
9.2	-	11.0	----------+
11.0	-	13.2	----------+
13.2	-	15.8	----------+
15.8	-	18.9	----------+
18.9	-	22.6	----------+
22.6	-	27.1	----------+
27.1	-	32.4	----------+
32.4	-	38.8	----------+
38.8	-	46.4	-----+
46.4	-	55.6	---+
55.6	-	66.6	--+
66.6	-	79.7	-+
79.7	-	95.5	+
95.5	-	114.3	+
114.3	-	136.8	+
136.8	-	163.8	+
163.8	-	196.2	+
196.2	-	234.9	+
234.9	-	281.2	+
281.2	-	336.7	+

TABLE No. 5

MALVERN AUTOSIZER II c for ethosomal system No. 510 (see Table 2)

Sample: 510; Vortex + US; Non diluted
Data from accumulation file sum of 6 blocks
Temperature: 22.6°C; Viscosity: 4.6400; Refractive Index: 1.374;
Angle: 90.0

Particle size distribution (nm.)			Graph of Distribution of mass
17.4	-	21.1	+
21.1	-	25.6	+
25.6	-	31.0	+
31.0	-	37.6	+
37.6	-	45.5	----------+
45.5	-	55.1	----------+
55.1	-	66.8	+
66.8	-	80.9	+
80.9	-	98.0	+
98.0	-	118.8	+
118.8	-	143.9	+
143.9	-	174.3	+
174.3	-	211.2	+
211.2	-	255.9	+|
255.9	-	310.0	+
310.0	-	375.6	+
375.6	-	455.0	----------+
455.0	-	551.3	----------+
551.3	-	667.9	----------+
667.9	-	809.2	----------+
809.2	-	980.3	----------+
980.3	-	1187.7	----------+
1187.7	-	1438.9	----------+
1438.9	-	1743.3	----------+

References

1. Barry, B.W., Optimizing percutaneous absorption. In Bronaugh, R.L. and Maibach, M.I. (Eds), Percutaneous Absorption, Dekker, New York, 1989, pp. 531-565.

2. Batzri, S. and Korn, E.D. Single bilayer liposomes prepared without sonication. Biochim. Biophys. Acta 298 1973) 1015-1019.

3. Kremer, J. M. H., v. d. Esker, M. W. J., Pathmamanoharan, C. and Wiersema, P. H. Vesicles of variable diameter prepared by a modified injection method. Biochemistry 16 (1977) pp.3932-3935.

4.Leigh, S., Pro-liposome compositions,United States Patent No.5,004,611, April 2, 1991.

5. Riaz, M., Weiner, N., Martin, F. In Pharmaceutical Dosage Forms. Disperse Systems, Vol. 2; Lieberman, H.A., Rieger, M.M., Banker, G.S., Eds, Marcel Dekker, Inc. New York and Basel, 1988 pp 567-602.

6. Touitou, E. and Fabin, B. Altered skin permeation of a highly lipophilic molecule: tetrahydrocannabinol. Int. J. Pharm. 43 (1988) 17-22.

7. Touitou, E., Levi-Schaffer, F., Shaco-Ezra, N., Ben-Yossef, Ramy and Fabin, B. Enhanced permeation of theophylline through the skin and its effect on fibroblast proliferation. Int. J. Pharm. 70 (1991) 159-166.

CLAIMS:

1. A liposomal composition for medical or cosmetic use, for topical application to the skin, resulting in the enhanced transdermal passage or introduction of an active ingredient into the skin, which composition contains vesicles in the size range from the nm range to the μm range, which composition comprises from 0.5% to 10% phospholipids, from 20% to 50% of a C_2 to C_4 alcohol, from 0 to 30% glycol, at least 20% water and at least one active ingredient where the combined alcohol and glycol content does not exceed 70%.

2. A composition according to claim 1, where the penetration through the skin is at least four times that of a similar liposome composition containing less than 5% alcohol.

3. A composition according to any of claims 1 or 2, where the phospholipids comprise at least one member selected from the group consisting of phosphatidylcholine, (P C), hydrogenated P C, phosphatidic acid (P A), phosphatidylserine (P S), phosphatidyl-ethanolamine (P E), phosphatidylglycerol (P P G) , phosphatidylinositol (P I), hydrogenated P C and similar compounds.

4. A composition according to any of claims 1 to 3, where the alcohol compound is ethanol, and where the mixture of such compounds comprises ethanol and propylene glycol, where the ethanol concentration in the final preparation is between 20 and 50 weight-% of the final product, the content of water being at least about 20 % w/w.

5. A composition according to any of claims 1 to 4, in which the active ingredient is a member selected from the group consisting of peptides, enzymes, hormones, anti-aging agents, tanning agents,

vitamins, antiviral drugs, plant extracts, glycosides, alkaloids, anxiolytics, antiepileptics, antifungals, non-steroidal anti-inflammatory drugs, antihypertensive agents, corticosteroids, minoxidil, cannabinoids, , antibiotics, hydroxy acids, antimitotics, antimycotics, retinoic acid, diclofenac and acyclovir.

6. A composition according to any of claims 1 to 5, where the alcohol is selected from ethanol and isopropyl alcohol, where the glycol is propylene glycol or ethyl diglycol (Transcutol, Gatte fosse, France), or a mixture of any of the alcohols with any of the glycols, or each alcohol by itself.

7. A composition according to any of claims 1 to 6, comprising 22 to 70% of a combination of the alcohol and propylene glycol, and more than 20% water.

8. A process for the production of a cosmetic or medical composition for topical application to the skin, for rapidly introducing into the skin, or for enhanced penetration through the skin, which contains an active ingredient in a carrier defined in any of claims 1 to 7 which comprises mixing a phospholipid, a C_2-to C_4-alcohol, or such alcohol and lower glycol, water, and an active ingredient so as to form a colloid system containing vesicles.

9. A composition according to any of claims 1 to 8, where the alcohol is ethanol, and where the mixture of such compounds comprises ethanol and propylene glycol, where the amount of ethanol is between 20 and 50 weight-% of the composition, the content of water being at least about 20 % w/w.

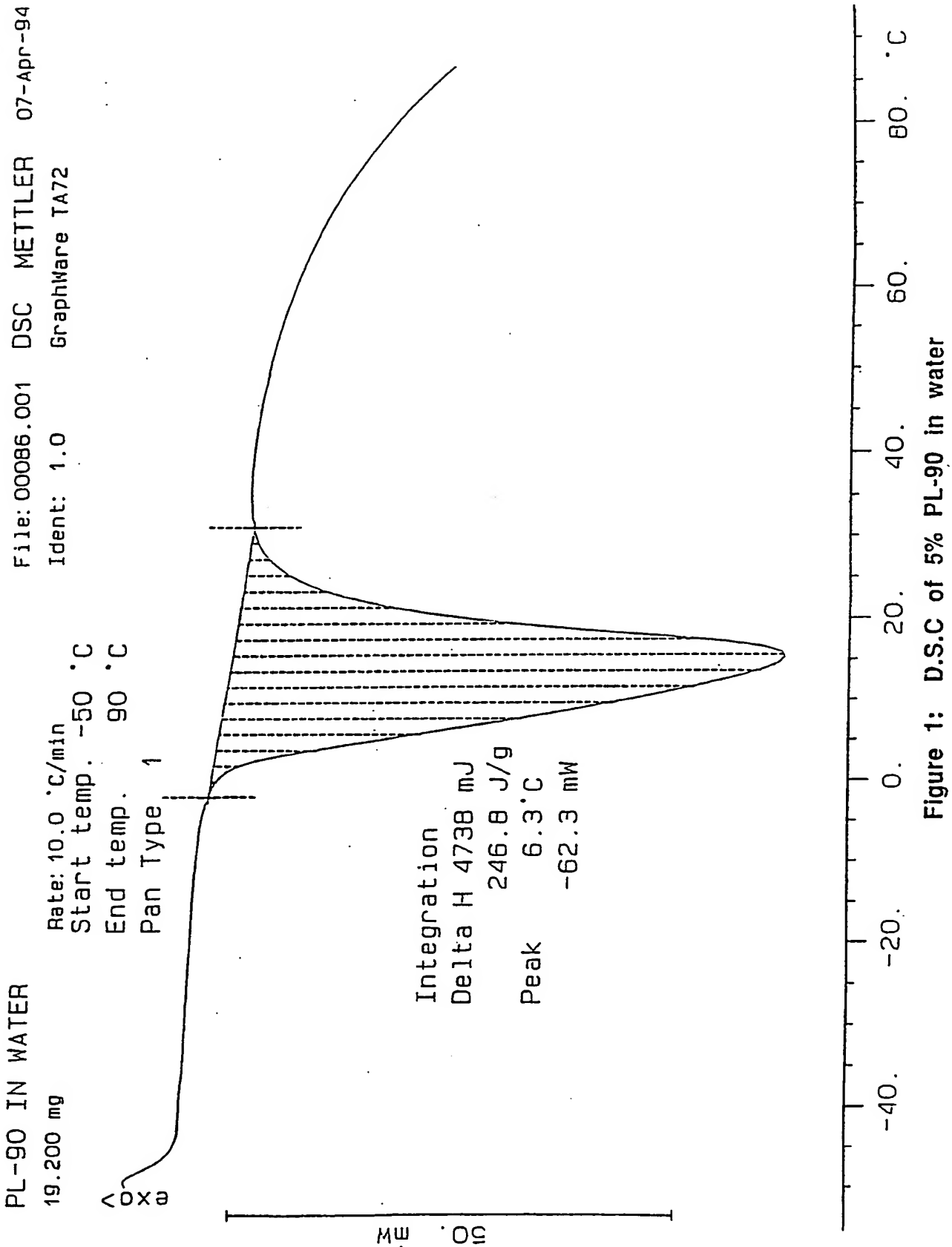

Figure 1: D.S.C of 5% PL-90 in water

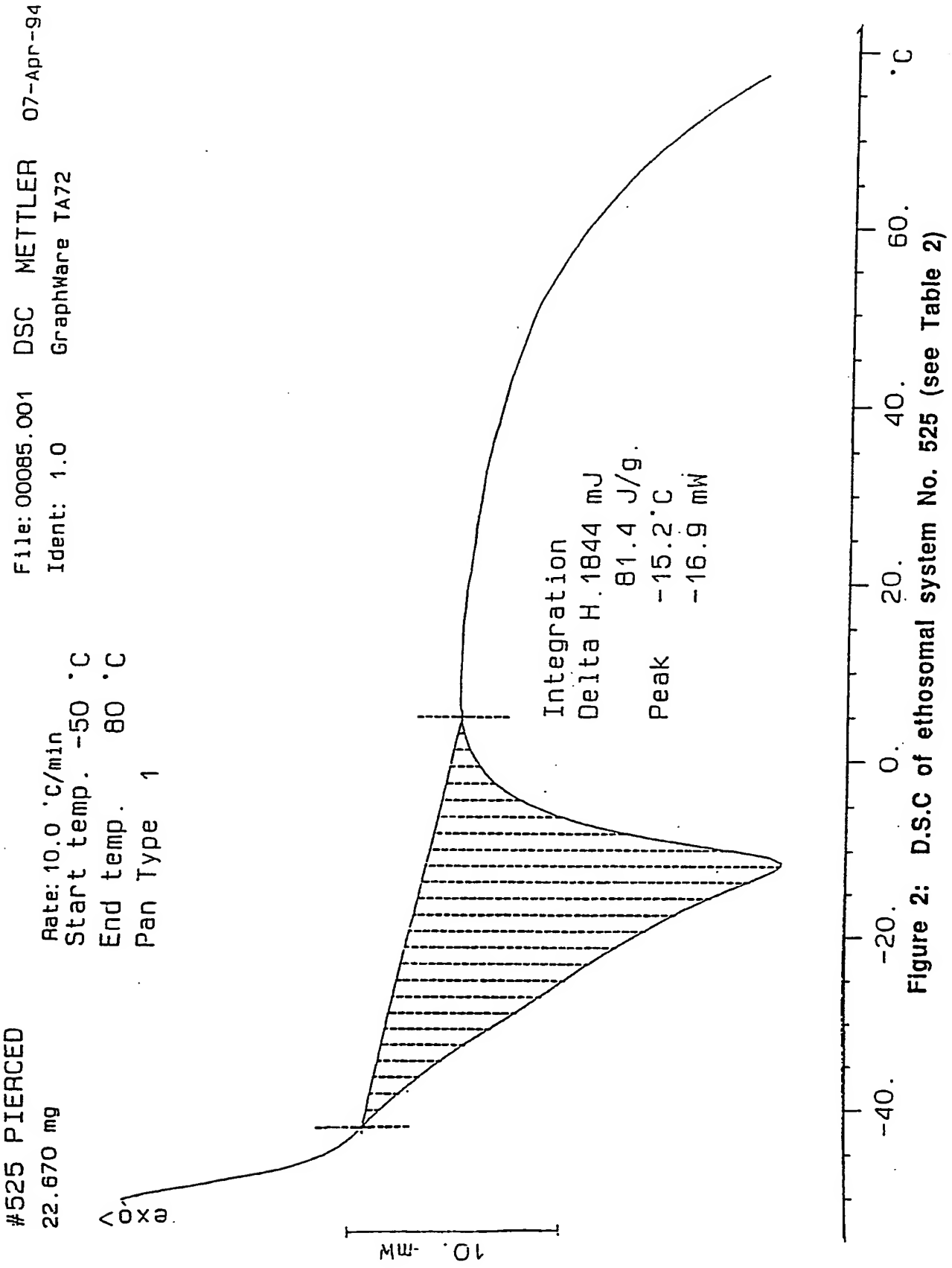

Figure 2: D.S.C of ethosomal system No. 525 (see Table 2)

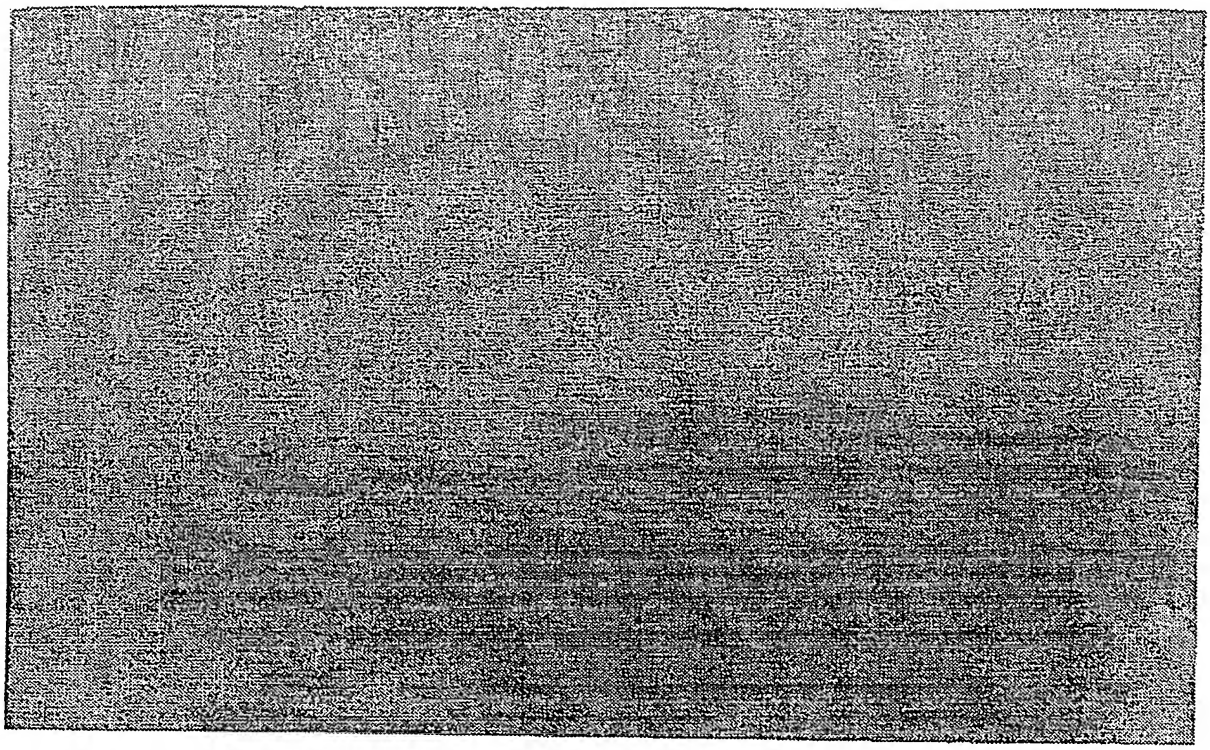

FIG. 3

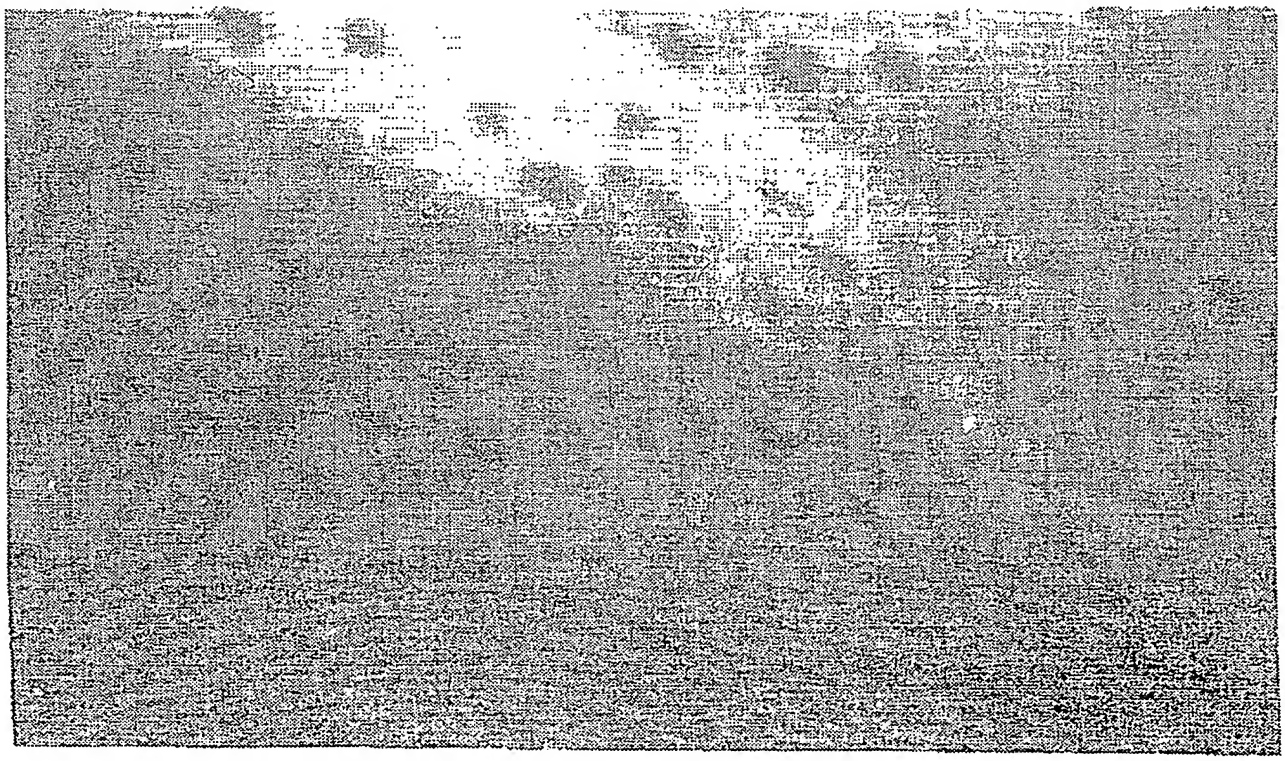

FIG. 4

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 95/02397

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/127 A61K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 582 239 (RHONE-POULENC RORER GMBH) 9 February 1994 see page 4, line 5 - line 15 see page 13; example 16	1-9
X	WO,A,91 11993 (NATTERMANN, A&CIE. GMBH) 22 August 1991 see page 13; example 1 see page 16; example 2 see claims 1-8	1-3,5
X	WO,A,92 18103 (PHARES PHARMACEUTICAL HOLLAND B.V.) 29 October 1992 see the whole document	1-9
Y	US,A,3 957 971 (OLENIACZ) 18 May 1976 see column 13; example 18	1-9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
21 September 1995	03. 10. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Benz, K

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INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/EP 95/02397

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SÖFW- SEIFEN, ÖLE, FETTE, WACHSE, vol. 114, no. 7, 21 April 1988 AUGSBURG (DE), pages 234-237, S. METJE ET AL. 'HERSTELLUNG UND VERMESSUNG VON LIPOSOMEN' see page 236, column 2, paragraph 5 ---	1-9
A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 81, no. 2, February 1992 WASHINGTON (US), pages 131-134, XP 000248775 E. TOUITOU ET AL. 'DYPHYLLINE LIPOSOMES FOR DELIVERY TO THE SKIN' see page 131, column 2, paragraph 4 ---	1-9
A	EP,A,0 177 223 (MEZEI) 9 April 1986 see the whole document -----	1-9

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 95/02397

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-582239	09-02-94	DE-A-	4225697	10-02-94
		DE-A-	4323174	12-01-95
		AU-B-	4697393	03-03-94
		CA-A-	2120511	17-02-94
		CN-A-	1084742	06-04-94
		WO-A-	9403156	17-02-94
		PL-A-	302979	05-09-94
WO-A-9111993	22-08-91	DE-A-	4003782	14-08-91
		DE-A-	4003783	14-08-91
		CA-A-	2067807	09-08-91
		DE-D-	59100466	11-11-93
		EP-A,B	0514435	25-11-92
		ES-T-	2060365	16-11-94
		JP-T-	5502882	20-05-93
		SG-A-	15294	10-06-94
WO-A-9218103	29-10-92	NONE		
US-A-3957971	18-05-76	CA-A-	1045979	09-01-79
EP-A-177223	09-04-86	JP-B-	6015467	02-03-94
		JP-A-	61085312	30-04-86
		US-A-	4897269	30-01-90
		US-A-	4761288	02-08-88